The Microbiological Contamination of Meteorites; A Null Hypothesis. A.Steele[1,2], J. K. W. Toporski[2], F. W. Westall[2], K. Thomas-Keprta[2], E. K. Gibson[2], R. Avci[4], C. Whitby[3], C. Griffin[3], D. S. McKay[1], [1]SEEPS, Astrobiology Group, University of Portsmouth, Portsmouth PO1 3QL, UK (andrew.steele@easynet.co.uk), [2]Mail Code SN, JSC, Houston, TX 77058, [3]University of Liverpool, Department of Biological Sciences, Liverpool, UK; [4]ICAL, Department of Physics, Montana State University, EPS 264, Bozeman, MT 59717, USA.

Since the revelation that terrestrial microorganisms reside in meteorites we have developed a 4 technique approach for the detection and analysis of these organisms, in combination with a large terrestrial control sample set [1]. This approach and the analysis thus far conducted have led this team to rethink the basic tenet of meteorite research, namely that although terrestrial contamination is a problem, this can be accounted for during analysis. This team believes that this hypothesis may not be as valid as previously thought. The four technique areas thus far utilised are as follows: Microscopy (SEM, FEGSEM and AFM), surface analysis (Time of Flight Secondary Ion Mass Spectrometry (ToFSIMS)), microbial culturing (using biochemical and 16srRNA identification) and direct isolation of DNA. Microscopy – Investigations of ALH84001, Nakhla, Murchison and the Antarctic chondrite ALH76004 have all shown the presence of organisms beneath the fusion crust. Toporski et al., (1999, 2000) have shown that, in the case of Nakhla, the meteorite is contaminated through to the center with both cellular structures and what appears to be exopolymeric secretions. In some cases the organisms are still actively growing [2,3]. ToFSIMS of all the above meteorites and a terrestrial control sample set has shown the presence of certain potential biomarker peaks in the mid 400 AMU range (Fig 1). These biomarkers appear only where there are bacteria and bacterial polymers present or suspected. The biomarkers occur in fresh bacterial biofilms, ALH84001, Nakhla, Murchison, terrestrial sediment, Antarctic cryptoendoliths, known bacterial fossils and Columbia River basalt. In all cases, controls used to test the technique and sample preparation did not show the biomarker peaks in the mid 400 AMU range. After the ToFSIMS investigation all samples were imaged with a FEG-SEM in an attempt to tie together the spectral data with morphological features. This has shown that certain other characteristic peaks in the mid 500 AMU range can be correlated to cellular features (Figure 2).

The use of simple culturing methods has produced viable identifiable organisms from Nakhla, ALH84001, Murchison, Allende and 2 Antarctic chondrites (ALH76004 and ALH81251). Interestingly in the case of Allende, of the species that have thus far been identified, two *Pseudomonads* (*P. capita and P. auricularis*) are found in the eye brows and behind the ears of humans [4]. Direct DNA analysis of an Allende sample has revealed that intact sequencable DNA can be extracted from meteorites using techniques developed to screen soil microbial populations. This technique differentiates between bacterial and fungal populations [4]. Experiments are continuing at this moment on ALH84001 and Nakhla with promising initial results.

In a further twist to the meteorite experiment, analysis conducted on organic rich fossils from the Oligocene Enspel Formation in central Germany has shown that after splitting and exposing the fossils to air, an extremely dense growth of contaminating fungi was observed after only a few months.

The implications of this research are underline the rapidity of contamination and the care necessary in sample preparation. Including the identification of mid 400 AMU range potential biomarkers by ToFSIMS, 9 out of 9 meteorites analysed are contaminated to varying degrees. These observations lead to several very simple questions: (1) Why are the organisms there?. The obvious answer is that the meteorites are providing food in the form of organic molecules. (2) How quickly do meteorites become contaminated?. The experiment on the organic rich fossil has shown that within 6 months there can be growth visible to the naked eye. (3) What is the effect of microbial contamination?. This is the crux of this abstract. The organisms thus far isolated can be found in both soils and on human beings. The soil microorganisms contribute to terrestrial weathering and therefore it is probable that the micro-organisms in the meteorites are doing the same thing, ie. making soil.

We therefore, propose that a new slant be taken on meteorite research, namely, to begin with the assumption that apart from some low molecular weight organic material (which must be present for the organisms to utilise as food), all other compounds could be the products of microbial and terrestrial contamination. With the wealth of literature available on the extraterrestrial nature of organics in meteorites this may seem improbable. The Murchison meteorite has been the source of much of our knowledge of extraterrestrial organics, however, the initial samples of this meteorite were collected some 4 – 6 months after Murchison had landed [5]. We have shown that this is plenty of time for an organic rich substrate to become heavily contaminated. There appears to be 4 main lines of evidence that show extraterrestrial origin; C^{13} measurements, deuterium, analysis, the presence of the so called extraterrestrial amino acids AIB, isovaline and norvaline, and racemic mixtures of some

of the proteinaceous amino acids. However, in the case of C^{13} and deuterium the organisms as they grow would take on the isotopic signature of the food source. In the case of excess deuterium in the kerogenaic material, this could be explained by the microbes use of proton pumps which remove intracellular protons and actively pump them into the high molecular weight extracellular matrix outside the cell. These pumps can increase 3-5 fold when pumping D out of the cell [6]. The external matrix is dominated by carbohydrate residues which have many available NH_2, OH and COOH groups. These groups are known to swap H for D extremely rapidly in the environment. Therefore the excess of deuterium in kerogenic material (compared with the low molecular weight organics) could be due to microbial action [7]. AIB isovaline and norvaline are both known to be extensively used in biogenic processes with AIB being present in ATP synthase, a ubiquitous molecule contained in every cell producing ATP. Indeed all of these amino acids have 3 base codons which are modified for inclusion of these residues into proteins [8]. Finally research conducted by Glavin et al (1999) on Nakhla show that this meteorite has the same amino acid profile as the sediment it was removed from and which must contain a wealth of microbial life, yet Alanine was present as almost a racemic mixture [9]. Indeed, in the last few years upwards of 70 – 80 new proteinaceous and non proteinaceous amino acids have been classified and the role of racemisation is still unclear [10].

In the light of the above research we, therefore, propose a null hypothesis, that all the higher weight macromolecules are the result of terrestrial contamination, to deal with this new evidence. Microbes in the meteorites could also mediate non-organic changes. In reality, the situation is probably a point somewhere between this null hypothesis and what we currently understand.

References: [1]. Steele et al. (1999) 30th LPSC Abstract. [2]. Toporski et al. (1999) 30th LPSC Abstract. [3]. Toporski et al. (2000) 31st LPSC Abstract. [4]. Whitby et al. (2000) 31st LPSC Abstract. [5]. Kvenholden et al. (1970) *Nature* 228. 923–926. [6]. Urrutia Mera et al. (1992). *Appl. Environ. Microbiol.* 58 (12). 3837–3844. [7]. Fogel and Cifuentes (1993). *Organic Geochemistry.* Engel and Macko Eds. Plenum Press. N.Y. [8]. Murzin et al. (1995). A structural classification of proteins database. *J. Molec Biol.* 247 :536–540. [9]. Glavin, D.P., et al. (1999), *Proc. Natl. Acad. Sci USA*, 96, 8835–8838. [10]. Singh B. J., ed. (1999). *Plant Amino Acids: Biochemistry and Biotechnology.* Marcel Dekker Inc., NY.

Figure 1. ToFSIMS spectra 300 – 500 AMU of ALH84001, Murchison (Mu), Cryptoendoliths (TP), Sediment (IOM), Columbia river basalt (CRB) and a fresh *Pseudomonas* biofilm.

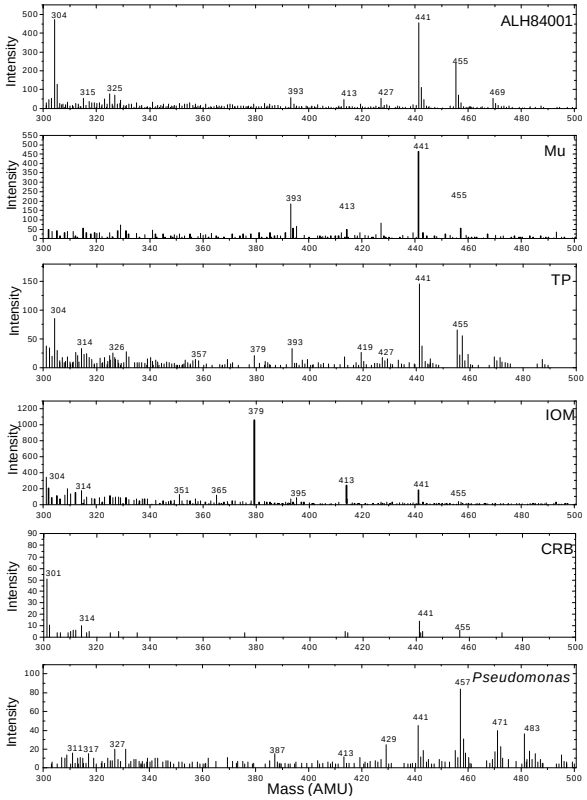
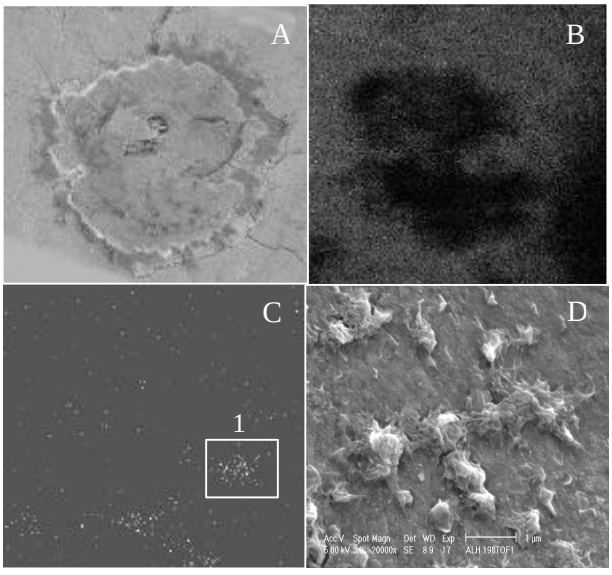


Figure 2. A) BSE image of a carbonate globule from ALH84001. B) ToFSIMS Si map of same globule. C) Map of distribution of peaks at 533 AMU. D) SEM images of cellular structures found in box 1 of Fig 2C.



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